

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP00/05930

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.⁷ C12N15/09

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl.⁷ C12N15/00-15/90

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
BIOSIS/MEDLINE/WPI (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GENOMICS, 49, May 1998 Vincent J. Kidd et al., "Duplication of the DR3 gene on human chromosome 1p36 and its deletion in human neuroblastoma", pp.385-393	1-5
A	Proc. Natl. Acad. Sci. USA, 92(12), June 1995 Peter S. White et al., "A region of consistent deletion in neuroblastoma maps within human chromosome 1p36.2-36.3", pp.5520-5524	1-5
A	Oncogene, 8(12), December 1993 Barker PE et al., "Translocation junctions cluster at the distal short arm of chromosome 1(1p36.1-2) in human neuroblastoma cells.", pp.3353-3358	1-5
A	Cancer Lett, 130(1-2), August 1998 Iolascon A. et al., "Interstitial and large chromosome 1p deletion occurs in localized and disseminated neuroblastomas and predicts an unfavourable outcome", pp.83-92	1-5

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent familyDate of the actual completion of the international search
22 November, 2000 (22.11.00)Date of mailing of the international search report
05 December, 2000 (05.12.00)Name and mailing address of the ISA/
Japanese Patent Office

Authorized officer

Facsimile No.

Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP00/05930

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	Oncogene, 19(37), 31 August, 2000, pp.4302-4307 Nakagawara A et al., "Identification and characterization of a 500-kb homozygously deleted region at 1p36.2-36.3 in a neuroblastoma cell line", pp.4302-4307	1-5

A. 発明の属する分野の分類 (国際特許分類 (IPC))

Int. Cl⁷ C12N15/09

B. 調査を行った分野

調査を行った最小限資料 (国際特許分類 (IPC))

Int. Cl⁷ C12N15/00-15/90

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用した電子データベース (データベースの名称、調査に使用した用語)

BIOSIS/MEDLINE/WPI (STN)

C. 関連すると認められる文献

引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する 請求の範囲の番号
A	GENOMICS, 49, May 1998 Vincent J. Kidd et al., "Duplication of the DR3 gene on human chromosome 1p36 and its deletion in human neuroblastoma", p. 385-393	1-5
A	Proc. Natl. Acad. Sci. USA, 92(12), Jun. 1995 Peter S. White et al., "A region of consistent deletion in neuroblastoma maps within human chromosome 1p36.2-36.3", p. 5520-5524	1-5

☒ C欄の続きにも文献が列挙されている。☐ パテントファミリーに関する別紙を参照。

* 引用文献のカテゴリー

- 「A」 特に関連のある文献ではなく、一般的技術水準を示すもの
「E」 国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
「L」 優先権主張に疑義を提起する文献又は他の文献の発行口若しくは他の特別な理由を確立するために引用する文献 (理由を付す)
「O」 口頭による開示、使用、展示等に言及する文献
「P」 国際出願日前で、かつ優先権の主張の基礎となる出願

の日の後に公表された文献

- 「T」 国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「X」 特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Y」 特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「&」 同一パテントファミリー文献

国際調査を完了した日

22.11.00

国際調査報告の発送日

05.12.00

国際調査機関の名称及びあて先

日本国特許庁 (ISA/JP)

郵便番号100-8915

東京都千代田区霞が関三丁目4番3号

特許庁審査官 (権限のある職員)

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電話番号 03-3581-1101 内線 3448

C (続き) . 関連すると認められる文献		
引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する 請求の範囲の番号
A	Oncogene, 8(12), Dec. 1993 Barker PE et al., "Translocation junctions cluster at the distal short arm of chromosome 1(1p36.1-2) in human neuroblastoma cells.", p. 3353-3358	1 - 5
A	Cancer Lett, 130(1-2), Aug. 1998 Iolascon A. et al., "Interstitial and large chromosome 1p deletion occurs in localized and disseminated neuroblastomas and predicts an unfavourable outcome", p. 83-92	1 - 5
T	Oncogene, 19(37), Aug. 31 2000, p. 4302-4307 Nakagawara A et al., "Identification and characterization of a 500-kb homozygously deleted region at 1p36.2-36.3 in a neuroblastoma cell line", p. 4302-4307	1 - 5

(12)特許協力条約に基づいて公開され、国際出願

(19) 世界知的所有権機関
国際事務局



(43) 国際公開日
2001年3月8日 (08.03.2001)

PCT

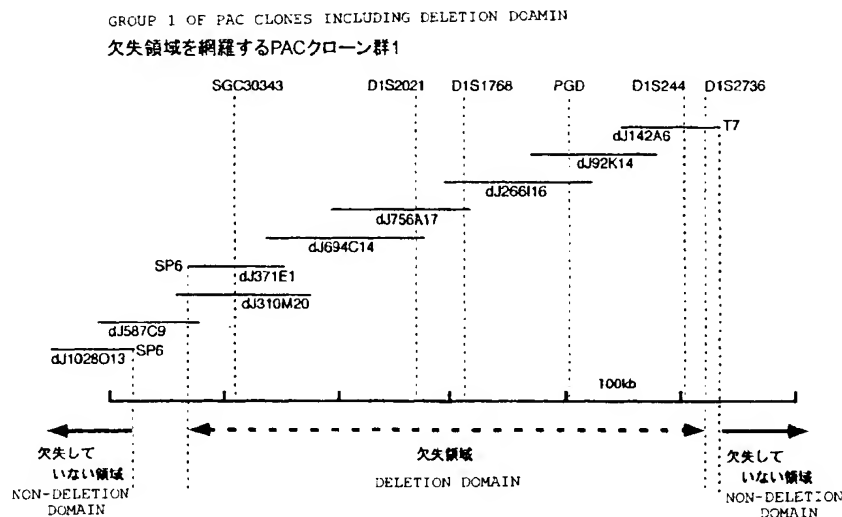
(10) 国際公開番号
WO 01/16311 A1

- (51) 国際特許分類: C12N 15/09 INC.) [JP/JP]: 〒841-0017 佐賀県鳥栖市田代大官町408 Saga (JP). 千葉県 (CHIBA-PREFECTURE) [JP/JP]: 〒260-8667 千葉県千葉市中央区市場町1番1号 Chiba (JP).
- (21) 国際出願番号: PCT/JP00/05930
- (22) 国際出願日: 2000年8月31日 (31.08.2000) (72) 発明者; および (75) 発明者/出願人 (米国についてのみ): 中川原章 (NAK-AGAWARA, Akira) [JP/JP]: 〒260-0801 千葉県千葉市中央区仁戸名町662番2-203 Chiba (JP).
- (25) 国際出願の言語: 日本語
- (26) 国際公開の言語: 日本語 (74) 代理人: 長谷川芳樹, 外 (HASEGAWA, Yoshiki et al.): 〒104-0061 東京都中央区銀座二丁目6番12号 大倉本館 創英国際特許法律事務所 Tokyo (JP).
- (30) 優先権データ: 特願平11/245962 1999年8月31日 (31.08.1999) JP (81) 指定国 (国内): AU, CA, JP, KR, US. 特願2000/136266 2000年5月9日 (09.05.2000) JP
- (71) 出願人 (米国を除く全ての指定国について): 光久製薬株式会社 (HISAMITSU PHARMACEUTICAL CO., (84) 指定国 (広域): ヨーロッパ特許 (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

[続葉有]

(54) Title: HUMAN 1p36 HOMOZYGOSITY DELETION DOMAIN

(54) 発明の名称: ヒト 1p36 ホモ接合性欠失領域



(57) Abstract: Base sequence data at the 36-position of the 1st chromosome short arm in human neuroblastoma cell lines NB-1 and MASS-NB-SCH-1, which are tumor suppressor genes in human neuroblastoma, are disclosed.

(57) 要約:

ヒト神経芽細胞腫の癌抑制遺伝子である、ヒト神経芽細胞腫細胞株NB-1、MASS-NB-SCH-1における第1番染色体短腕36位の欠失領域の塩基配列情報が開示される。

WO 01/16311 A1



添付公開書類:
— 国際調査報告書

2文字コード及び他の略語については、定期発行される各PCTガゼットの巻頭に掲載されている「コードと略語のガイダンスノート」を参照。

明細書

ヒト 1 p 3 6 ホモ接合性欠失領域

技術分野

5 本発明は、ヒト神経芽細胞腫の第 1 番染色体短腕 3 6 位（以下、1 p 3 6 ともいう）に共通に存在するホモ接合性欠失（Homozygous Deletion）領域（以下、単に欠失領域ともいう）に関する。

背景技術

10 発癌に関与している癌関連遺伝子には、不活性化されて発癌を促す癌抑制遺伝子が存在することが知られており、例えばRBまたはWT-1という癌抑制遺伝子がすでに見いだされ同定されている。

RB遺伝子は小児の眼の癌である網膜芽細胞腫（Retinoblastoma）の原因となる遺伝子として発見されたためこの名が付けられた。網膜芽細胞腫ではヒト第 1 3 染色体長腕 1 4 領域（1 3 q 1 4）が短くなっていることが多く（Yunis J J. 等 Am. J. Dis. Child. 132, 161
15 -163（1978））、この部分にある遺伝子が失活すると癌になりやすくなるのではないかと以前から推定されていた。しかし遺伝子のクローニングは難しく、1986年になってヒトの癌抑制遺伝子としては初めてRBが同定されるに至った。（Friend S H. 等 Nature 323, 643-646（1986））

20 ウイルムス腫瘍（Wilms' Tumor）は小児の後腎造腎組織より発生する胎児性悪性腫瘍で、腎芽腫とも呼ばれる。患者はしばしば泌尿生殖器の異常を伴うが、疫学的データから網膜芽細胞腫と同様に“Two-hit-theory”が適用できるとされ、劣性型の腫瘍遺伝子の関与が推定されていた。腫瘍（W）と泌尿生殖器（G）の異常に加えて、無虹彩症（A）と精神発達遅滞（R）
25 を伴うWAGR症候群の患者で第 1 1 番染色体短腕 1 3 領域（1 1 p 1 3）に微小な欠失がしばしば認められる。この共通欠失領域に位置する遺伝子としてWT

1が単離された (Ca11 KM. 等 Cell 60, 509-520 (1990))。

その後も染色体の欠失領域の情報を元に多くの癌抑制遺伝子が発見されたが、1 p 3 6 の欠失領域から神経芽細胞腫の癌抑制遺伝子は現在もなお同定されるに至っていない。

発明の開示

本発明は、ヒト神経芽細胞腫に共通に存在する第1番染色体短腕36位の新規なホモ接合性欠失領域に関する情報を提供することを目的とする。

さらに、該領域の塩基配列情報に基づき、腫瘍の診断、予後同定の方法およびそのために使用可能な腫瘍マーカーを提供することを目的とするものである。

また、該塩基配列情報に基づき、遺伝子診断方法、抗癌剤の開発、腫瘍病体メカニズムの解析方法、腫瘍病体メカニズムの解析に用いる試薬の開発を提供することを目的とする。

本発明者は、神経芽細胞腫の癌抑制遺伝子を見いだすべく鋭意研究した結果、ヒト神経芽細胞腫の細胞株であるNB-1の第1番染色体短腕36位において、新規なホモ接合性欠失領域を見いだした。

かかる知見に基づき、腫瘍の診断、予後同定の方法およびそのために使用可能な腫瘍マーカーを設計することを可能とし、本発明を完成した。さらに、該領域の塩基配列情報に基づき、腫瘍の遺伝子診断方法、抗癌剤の開発、腫瘍病体メカニズムの解析方法、腫瘍病体メカニズムの解析に用いる試薬の開発を可能とし本発明を完成した。

より詳しくは、本発明は、ヒト神経芽細胞腫の第1番染色体短腕36位に共通に存在するホモ接合性欠失領域にかかるものである。

より詳しくは、前記領域が、少なくともPACクローンであるdJ1028013のSP6側プライマーセットとPACクローンであるdJ371E1のSP6側プライマーセットとの間から、プライマーセットD1S2736とPACク

ローンである d J 1 4 2 A 6 の T 7 側プライマーセットとの間で規定されることを特徴とする領域にかかるものである。

また、前記領域が、さらに少なくともプライマーセット S G C 3 0 3 4 3 および D 1 S 2 7 3 6 で規定されることを特徴とする領域にかかるものである。

5 また、前記領域がヒト細胞芽細胞種の細胞株 N B - 1 及び M A S S - N B - S C H - 1 株で欠失していることを特徴とする領域にかかるものである。

また、本発明は、前記領域から得られる塩基配列情報に基づく、腫瘍マーカー、腫瘍の診断試薬、腫瘍の予後同定試薬、及び抗癌剤を含むものである。

10 さらに、本発明は、前記領域の塩基配列情報に基づく、腫瘍病体メカニズムの解析方法、及び腫瘍病体メカニズムの解析試薬を含むものである。

図面の簡単な説明

図 1 は、D 1 S 5 4 8、D 1 S 2 4 4 プライマーセットを用いたヒト神経芽細胞腫ゲノム DNA に対する P C R 反応物の 4 % アガロースゲルによる電気泳動写真である。ここで、レーン 1 : 分子量マーカー (上から 1 3 3 0, 8 2 8, 7 5 0, 6 9 6, 5 3 6, 5 1 7, 5 0 1, 3 9 6, 3 5 1, 2 2 1, 7 5, 6 5, 3 5, 2 2 b p)、レーン 2 : S K - N - B E、レーン 3 : S K - N - D Z、レーン 4 : R T - B M 1、レーン 5 : N L F、レーン 6 : L A - N - 5、レーン 7 : I M R 3 2、レーン 8 : N M B、レーン 9 : O A N、レーン 1 0 : N G P、レーン 1 1 : N B 1、レーン 1 2 : C H P 1 3 4、レーン 1 3 : K C N、レーン 1 4 : N B L - S、レーン 1 5 : C H P 9 0 1、レーン 1 6 : S Y 5 Y、レーン 1 7 : G A N B、レーン 1 8 : S K - N - A S、レーン 1 9 : K A N、レーン 2 0 : L H N、レーン 2 1 : S A N、レーン 2 2 : T G W、レーン 2 3 : 陽性対照 (ヒトリンパ球)、レーン 2 4 : M A S S - N B - S C H - 1、レーン 2 5 : 陰性対照 (滅菌蒸留水)、レーン 2 6 : 分子量マーカー (レーン 1 と同一) である。

25 図 2 は、D 1 S 2 0 2 1、P G D、S G C - 3 0 3 4 3、D 1 S 1 7 6 8、D 1 S 2 7 3 6 プライマーセットを用いた N B - 1、M A S S - N B - S C H - 1

ゲノムDNAに対するPCR反応物の2%アガロースゲルによる電気泳動写真である。なお全てのPCR反応物は陽性対照としてヒト神経増殖因子(human Neuro Growth Factor β : hNGF β)領域を特異的に増幅するプライマーを含んでいる。ここで、レーン1:空レーン、レーン2:分子量マーカー(上から1030, 734, 341, 258, 192, 105, 78, 75, 46, 36, 18, 17, 12, 11, 8bp)、レーン3:陽性対照(SY5Y)、レーン4:NB-1、レーン5:MASS-NB-SCH-1、レーン6:陰性対照(滅菌蒸留水)、レーン7:陽性対照(SY5Y)、レーン8:NB-1、レーン9:MASS-NB-SCH-1、レーン10:陰性対照(滅菌蒸留水)、レーン11:陽性対照(SY5Y)、レーン12:NB-1、レーン13:MASS-NB-SCH-1、レーン14:陰性対照(滅菌蒸留水)、レーン15:陽性対照(SY5Y)、レーン16:NB-1、レーン17:MASS-NB-SCH-1、レーン18:陰性対照(滅菌蒸留水)、レーン19:陽性対照(SY5Y)、レーン20:NB-1、レーン21:MASS-NB-SCH-1、レーン22:陰性対照(滅菌蒸留水)、レーン23:分子量マーカー(レーン2と同一)である。

図3は、D1S244プライマーセットを用いたPACのクローンDNAに対するPCR反応物の5%アクリルアミドによる電気泳動写真である。ここで、レーン1:分子量マーカー(1202bp、517bp、396bp、201bp)、レーン2~4:dJ142A6、レーン5~7:dJ332G17、レーン8~10:dJ369D13、レーン11:陽性対照(ヒト胎盤DNA)、レーン12:陰性対照(滅菌蒸留水)である。

図4は、1stスクリーニングによりNB-1、MASS-NB-SCH-1株において見いだされた1p36に含まれるPAC18クロンのコンティグ図である。

図5は、最終的にNB-1、MASS-NB-SCH-1株において見いださ

れた 1 p 3 6 に含まれる P A C 3 4 クローンのコンティグを示す図である。

図 6 は、P A C クローンの情報を元に設計したプライマーを用いた N B - 1、
M A S S - N B - S C H - 1 ゲノム D N A に対する P C R 反応物の電気泳動写真
である。ゲルは 2. 5 % アガロースを用いた。ここで、各レーンは以下のとおり
5 レーン 1 : マーカー (1 2 0 2 b p、5 1 7 b p、3 9 6 b p、2 0 1
b p)、レーン 2 : 陽性対照 (ヒト胎盤 D N A)、レーン 3 : 1 p 3 6 に欠損を有
さない神経芽細胞腫患者由来の正常細胞、レーン 4 : 1 p 3 6 に欠損を有さない
神経芽細胞腫患者由来の癌細胞、レーン 5 : M A S S - N B - S C H - 1 患者由
来の正常細胞、レーン 6 : M A S S - N B - S C H - 1、レーン 7 : N B 1、レ
10 レーン 8 : 陽性対照 (ヒト染色体を有するマウスハイブリドーマ)、レーン 9 : 陽性
対照 (ヒトリンパ球)、レーン 1 0 : 陽性対照 (ヒト胎盤 D N A)、レーン 1 1 :
1 p 3 6 に欠損を有さない神経芽細胞腫患者由来の正常細胞、レーン 1 2 : 1 p
3 6 に欠損を有さない神経芽細胞腫患者由来の癌細胞、レーン 1 3 : M A S S -
N B - S C H - 1 患者由来の正常細胞、レーン 1 4 : M A S S - N B - S C H -
15 レーン 1 5 : N B 1、レーン 1 6 : 陽性対照 (ヒト染色体を有するマウスハ
イブリドーマ)、レーン 1 7 : 陽性対照 (ヒトリンパ球)、レーン 1 8 : 空レーン。

また、A においては、レーン 1 ~ 8 はプライマーとして d J 1 0 2 8 O 1 3 -
S P 6 を用い、レーン 9 ~ 1 7 はプライマーとして d J 1 4 2 A 6 - T 7 を用い
た。B においては、レーン 1 ~ 8 はプライマーとして d J 3 7 1 E 1 - S P 6 を
20 用い、レーン 9 ~ 1 7 はプライマーとして d J 5 8 7 C 9 - S P 6 を用いた。

図 7 は、本発明により明らかとなった 1 p 3 6 におけるゲノム欠失領域範囲を
示す図である。

図 8 は、本発明により明らかとなった 1 p 3 6 におけるゲノム欠失領域範囲に
含まれる最小限の P A C クローン群の位置を示す図である。

25 図 9 は、本発明により明らかとなった 1 p 3 6 におけるゲノム欠失領域範囲に
含まれる、最小限の P A C クローン群の位置を示す図であり、図 8 の組み合わせ

と多少異なるものである。

発明を実施するための最良の形態

(1 p 3 6における新規ホモ接合性欠失領域)

本発明者等は、(1) ヒト1 p 3 6の欠失は、少なくとも14種類のヒト腫瘍
5 (大腸癌、乳癌、脳腫瘍など) において見出されており、さらにこの領域に複数
個のがん抑制遺伝子が存在することが予測され、その欠失は神経芽細胞腫の予後
の不良さと高い相関を示すこと (Schwab M. 等、Genes Chromosomes Cancer 16, 211-229 (1996)、Fong C. T. 等、Cancer Res. 52, 1780-1785 (19
10 92)) に注目し、また、大腸癌において正常1 p 3 6を導入することで癌細胞
の正常化が認められること (Tanaka K. 等、Oncogene 8, 2253-2258 (1993))、肝癌や大腸癌において1 p 3 6の欠失は癌の
初期段階から認められること (Kuroki T. 等、Genes Chromosomes Cancer 13, 163-167 (1995)) に注目し
15 、1 p 3 6の欠失が正常細胞の癌化の初期に関与する可能性が高いと考えた。

さらに、癌抑制遺伝子の同定はヘテロ接合性の喪失 (Loss Of Heterozygosity: 以下LOHともいう) の解析により可能である。すな
わち性染色体を除くヒト細胞の染色体は全て対になって存在し、遺伝子も母方からと父方からの2つのコピーが存在する。ここでKundson A. G. 等が
20 提唱した “two - hit theory” (Kundson A. G., Pediatr Res 10, 513- (1976)) によると、遺伝子の不
活性は母方、父方の2つのコピーが両方とも不活性化した場合にのみ観察される
こととなるが、このメカニズムでもっともよく観察されるのが前記LOHである
。ここでLOHとは、一方のコピーに小さな遺伝子異常 (塩基、遺伝子の一部の
25 欠失や置換) が存在し、もう一方のコピーが存在する染色体はその遺伝子を含ん
だ大きな領域がそっくり欠失するパターンを意味する。従って、癌抑制遺伝子の

不活性化により癌が発症した患者では、癌抑制遺伝子が存在する染色体が正常細胞では2コピー、腫瘍細胞では1コピーになっていると考えられている。

しかし通常観察される前記LOHの範囲は、分子遺伝子学的距離としてはきわめて長大で、その中に含まれる遺伝子の数及び塩基数は非常に膨大である。実際
5 現在1p36の欠失領域は、LOHの解析により1p36.2-1p36.3の約8~10Mbに狭められてはいるが、さらに領域の絞り込みが必要である。

そこで、本発明者らは、癌抑制遺伝子の同定のために、神経芽細胞腫瘍の細胞株を解析した結果、神経芽細胞腫瘍の染色体1p36に約700kbのホモ接合性欠失部分が認められることを見出した。さらにこの700kbは、ヘテロ接合性の喪失の解析により明らかにされていた欠失領域(約8~10Mb)の一部であった。
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(ヒト神経芽細胞腫1p36ホモ接合性欠失領域に関する応用例)

(1) 腫瘍の遺伝子診断、予後同定の方法およびそのために使用可能な腫瘍マーカー

15 本発明により開示された塩基配列をもちいて試料細胞中の1p36ホモ接合性欠失領域の有無を調べることにより、腫瘍の診断、予後同定が可能である。欠失領域の有無を調べるには、例えば1p36ホモ接合性欠失領域の任意の配列とハイブリダイズし得る塩基配列(プローブ)を使用する全ての方法が提供される。これらのプローブは、必要に応じてラジオアイソトープ、酵素(例えばアルカリ
20 フォスファターゼ、ペルオキシターゼ)、蛍光化合物(例えばフルオレセイン)、あるいは化学発光化合物(例えばアクリジニウムエステル、イソルミノール)等で標識される。すなわち試料細胞中にプローブ配列とハイブリダイズする核酸が存在しない場合、腫瘍発症の可能性が高く、既に腫瘍を発症している検体では予後が不良であると診断することが可能である。ハイブリダイズさせる方法としては例えば、サザンブロットィング、ノザンブロットィング、ドットブロットィ
25 ング、Fluorescence in situ hybridization

n (FISH) 法などが使用できる。

欠失領域の有無を調べるには、他にも 1 p 3 6 ホモ接合性欠失領域の任意の配列をプライマーとして Polymerase Chain Reaction (PCR) 法を用いることにより可能である。

5 ヘテロ接合性の喪失を同定するには、例えば Restriction Fragment Length Polymorphism (RFLP) 法を用いることにより可能である。

 このような方法は当事者にとって周知の方法によって行われる。例えば、Molecular Cloning A LABORATORY MANUAL (T. Maniatis 著: Cold Spring Harbor Laboratory Press 社)、遺伝子病入門 (高久史麿 著: 南江堂) を参照。

(2) 抗癌剤の開発

 本発明により開示された塩基配列をもちいて 1 p 3 6 ホモ接合性欠失あるいはヘテロ接合性の消失を有する細胞を見だし、抗癌剤のスクリーニングが可能である。これらの細胞は癌化しているため、細胞の分裂増殖が正常細胞に比較して早い。細胞の培養液中に抗癌剤の候補物質を添加し細胞の分裂増殖速度を観察することで 1 p 3 6 ホモ接合性欠失あるいはヘテロ接合性の消失を有する腫瘍の抗癌剤がスクリーニングできる。細胞の分裂増殖速度は例えば、ラジオアイソトープ (例えばトリチウム) ラベルしたチミジンの取り込み量を測定することにより可能である。

(3) 腫瘍病体メカニズムの解析方法

 本発明により開示された塩基配列をもちいて 1 p 3 6 ホモ接合性欠失あるいはヘテロ接合性の消失を有する細胞を見だし、腫瘍病体メカニズムの解析する事が可能である。これら細胞と正常細胞の蛋白質を抽出し、例えばウエスタンブロッティングにより正常細胞でのみ発現している蛋白質を同定することが可能である。その蛋白質を用いて例えばアフィニティークロマトグラフィーにより特異的

に相互作用する別の蛋白質の同定も可能である。これらの手法を繰り返すことにより発癌に関する蛋白質の同定しその細胞内における機能を解析することで腫瘍病体メカニズムの解析が可能である。

以下、本発明により見いだされた神経芽細胞腫に共通の 1 p 3 6 のホモ接合性
5 欠失領域について、実施例に即してさらに詳しく説明する。

実施例

(実施例 1) ヒト神経芽細胞腫からのゲノム DNA の抽出

以下のヒト神経芽細胞腫由来の細胞株をそれぞれ 1 0 c m の培養皿でほぼコンフルエントになるように培養した。ここで使用した各細胞名とその入手方法を以下にまとめた。

SK-N-BE (ATCC Number: CRL-2271)

SK-N-DZ (ATCC Number: CRL-2149)

RT-BM1

NLF: Garrett M Brodeur より供与

15 LA-N-5: (理研細胞バンク: RC0485)

IMR32: Garrett M Brodeur より供与

NMB: Garrett M Brodeur より供与

OAN: Garrett M Brodeur より供与

NGP: Garrett M Brodeur より供与

20 NB1: 京都府立医科大学小児科の松村隆文先生より供与 (新潟大学脳研究所で樹立された日本で最も古い神経芽細胞腫細胞株うちのひとつとされている)

CHP134: Garrett M Brodeur より供与

KCN: Garrett M Brodeur より供与

NBL-S: Garrett M Brodeur より供与

25 CHP901: 患者の手術摘出標本から細胞株樹立した

SY5Y: Garrett M Brodeur より供与

GANB : 患者の手術摘出標本から細胞株樹立した

SK-N-AS (ATCC Number : CRL-2137)

KAN : Garrett M Brodeurより供与

LHN : Garrett M Brodeurより供与

5 SAN : Garrett M Brodeurより供与

TGW : Garrett M Brodeurより供与

MASS-NB-SCH-1 : 静岡県立こども病院臨床病理科の平岩久幸先生より供与。Cancer 79, 2036-2044 (1997)

KP-N-NB : 京都府立医科大学小児科の松村隆文先生より供与

10 NB69 (理研細胞バンク : RC0480)

NBTu-1 : 日赤医療センター小児外科の横森欣次先生より供与

SK-N-MC (ATCC Number : HTB-10)

SK-N-SH (ATCC Number : HTB-11)。

15 得られたコンフルエントの培養皿2枚を用いてトリプシン処理により細胞を摂取した。摂取した細胞を5mlのTEN buffer (TEN : 50mM Tris-Cl (pH8.0)、1mM EDTA、100mM NaCl) 中に懸濁しホモジナイズした。ホモジナイズした懸濁液に750 μ lのSDS (10%) と125 μ lのproteinase K (MERCK社製、20mg/ml) を加え、緩やかに転倒混和し、50 $^{\circ}$ Cで一晩インキュベートして細胞を溶解させた。

20 翌日、5.1mlの飽和フェノールを加え、1時間混和し10,000rpmで10分遠心して上清を回収した。さらにもう一度、3,000rpmで10分遠心して上清を回収した。

25 回収した上清に5.1mlのクロロホルム : イソアミルアルコール = 24 : 1 を加えて1時間混和したあと、3,000rpmで10分遠心して上清を回収した。

回収した上清にあらかじめ -20°C で保冷した 10.2 ml のエタノールを加えて混和し、得られる糸状のDNAをパスツールピペットでとり、余分のエタノールを取り除いて乾燥させた。乾燥させたDNAに適量のTE buffer (Tris-Cl ($\text{pH } 8.0$), 1 mM EDTA) を加え、室温で $1\sim 2$ 日混和してDNAを溶解した。

(実施例2) ヒト神経芽腫細胞株から抽出したゲノムDNAの精製

ヒト神経芽腫細胞株から抽出したゲノムDNAをQIA amp Blood kit (QIAGEN社製) を用いて以下に示す条件で精製した。

抽出したゲノムDNAを 260 nm の紫外光の吸収により定量し、 $5\sim 10\mu\text{g}$ のDNAに適量のTE bufferを加え $225\mu\text{ l}$ とした。さらにエタノールを $210\mu\text{ l}$ 加え攪拌した。

攪拌液を付属のQIA ampカラム (キット付属品、QIAGEN社製) に移し、 8000 rpm で1分間遠心しゲノムDNAをカラムに吸着させた。カラムに $500\mu\text{ l}$ のBuffer AW (キット付属品、QIAGEN社製) を加えて 8000 rpm で1分間遠心した。さらにbuffer AWをいれて 15000 rpm で3分間遠心したあと、カラムを 1.5 ml の新しいチューブへ移し、あらかじめ 70°C に暖めておいたbuffer AEを $200\mu\text{ l}$ 加え、室温で1分間放置後、 8000 rpm で1分間遠心しDNAを精製した。

260 nm の紫外光の吸収により精製したゲノムDNAを定量した。

(実施例3) ヒト神経芽腫細胞ゲノムDNAを用いた1p36部位特異的PCRによる欠失株の同定

実施例1、2で抽出、精製した27種類のヒト神経芽細胞腫由来細胞株からの細胞のゲノムDNAを用いて、1p36の欠失を以下に示すような部位特異的PCRにより同定した。また、使用したPCRプライマーは、特に記載されない限り、サワディーテクノロジー社製のものをを用いた。

$1\mu\text{ l}$ のゲノムDNA溶液 ($2\text{ ng}/\mu\text{ l}$)、 $1\mu\text{ l}$ の $10\times\text{Pfu buf}$

fer、 $1\mu\text{l}$ のdNTPs (2mM)、 $0.2\mu\text{l}$ のD1S548F (20p
mole/ μl)、 $0.2\mu\text{l}$ のD1S548R (20p mole/ μl)、 0
 $.2\mu\text{l}$ のD1S244F (20p mole/ μl)、 $0.2\mu\text{l}$ のD1S24
4R (20p mole/ μl)、 $6.12\mu\text{l}$ の滅菌蒸留水、および 0.08μ
5 lのPfuを混合し、 $10\mu\text{l}$ の混合液を調製した。

ここでD1S548およびD1S244はともに1p36.23-31領域内
を特異的に増幅することが可能な既知のプライマーセットであり、それらのシー
ケンスを表1に示す。

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表 1

D1S548及びD1S244のシーケンス

プライマーセット	シーケンス(F) 5'-3' (配列番号)	シーケンス(R) 5'-3' (配列番号)	染色体の位置	増幅サイズ
D1S548	GAACTCATTGGCAAAAGGAA (25)	GCCICTTTTGTTCAGTGATT (28)	1p36.23-31	167bp付近
D1S244	GAGCAGCACCGTACAAAT (26)	AGCTCCGCTCCCCTGTAAT (7)	1p36.23-31	285-296bp付近

D1S548は、D1S244より1.3Mb染色体のテロメア側を増幅する。
F及びRはプライマーの方向を示している。

ここで、1p36.23-31領域内に全く欠失が存在しない場合、D1S548によって167塩基付近、D1S244によって285～296塩基付近に
5 増幅されたバンドが観察される。また本発明により特定された1p36.23-31領域内に欠失が存在する場合、D1S548によって167塩基付近のバンドは観察されるが、D1S244によって285～296塩基付近で増幅されたバンドは観察されない。

前記混合液を96℃で3分間変性させたあと、94℃で30秒間、56℃で4
10 5秒間、72℃で1分間を1サイクルとして30サイクル繰り返し、最後に72℃で10分間インキュベートしてPCR反応を行った。得られたPCR反応物を4%のアガロースゲルを用いて電気泳動した。得られた結果を図1に示す。

陽性コントロールのプライマーセットであるD1S548によって増幅される169塩基付近のバンドは全ての細胞株で観察された。それに対してD1S244によって増幅される285～296塩基付近のバンドはNB-1及びMASS-
15 NB-SCH-1株においてのみ観察されなかった。

(実施例4)

そこでNB-1及びMASS-NB-SCH-1の2株において1p36.23-31領域内を特異的に増幅することが知られている他の5つのプライマーセットSGC-30343、D1S2021、D1S1768、PGD、D1S2736についてもそれぞれPCRを行った。陽性対照としてヒト1番染色体短腕13位(1p13)に位置するヒト神経成長因子(human Nerve Growth Factor β :hNGF β)を2種類用いた。hNGF β 60sはアニーリング温度が60℃付近、hNGF β 57sはアニーリング温度が57℃
20 付近のプライマーで、それぞれ253bp、229bp付近にバンドが増幅される。これらのプライマーのシーケンスを表2に示す。

表 2

SHGC-31453、SGC-30343、DIS2021、DIS1768、PGD、DIS2736のシーケンス

プライマーセット	シーケンス(F) 5'-3' (配列番号)	シーケンス(R) 5'-3' (配列番号)	増幅サイズ
SHGC-31453	AAACCTCAGCAGTGGTCCIG(2)	AAACCCCTCTTCTTAGAGTGACG(1)	129bp付近
SGC-30343	GTGTTGGAGGCCAAATGTG(32)	TTGACCGGTGTTTGTCACTTAAA(39)	131bp付近
DIS2021	GTGAAGAGCCAGAGATGTCC(30)	CATAGTGCCCAACACGCAAG(15)	190bp付近
DIS1768	ATCTGATGTTTGGAGCTTGAG(13)	ACTAACAGCTCCGTGACGC(5)	147bp付近
PGD	TTCAGTTGATCAGAGTGCG(38)	ACCAGGAGCTGCTCATGTG(4)	131bp付近
DIS2736	CCTCCAGGGTATCTTGG(18)	TTTTTGAGGTGTGAGAGCAG(42)	130bp付近

この結果 (図2)、陽性対照であるhNGF β では増幅が予想されるバンドは検出されたが、1p36.23-31領域内で増幅が予想されるバンドが観察されなかった。

以上の結果から、ヒト神経芽細胞腫株であるNB-1及びMASS-NB-SC H-1は1p36の少なくともSGC-30343からD1S2736で規定される領域に両染色体の欠失を有するものと判断された。

(実施例5) 欠失領域のシーケンスを含むPACクローンを選択

実施例3で1p36.23-31領域の欠失を確認したプライマーセットD1S244を用いて、ヒトゲノムをランダムに有するPACクローン (P1-derived artificial chromosome: オリゴサービスつくば研究所製) をLA PCR kit (宝酒造社製) を用いて以下の方法でPCRを行い、欠失領域のシーケンスを含むPACクローンを選択を行った。

5 μ lのPAC DNA pool溶液 (Z1~Z4: オリゴサービスつくば研究所製)、2 μ lの10xLA PCR buffer、2.5 μ lのdNTPs (2mM)、0.4 μ lのD1S244F (100pmole/ μ l)、0.4 μ lのD1S244R (100pmole/ μ l)、9.6 μ lの滅菌蒸留水、および0.1 μ lのLA taq (5u/ μ l) を混合し20 μ lの混合液を調製した。

混合液を94 $^{\circ}$ Cで3分間変性させた後、94 $^{\circ}$ Cで30秒間、56 $^{\circ}$ Cで30秒間、72 $^{\circ}$ Cで1分間を1サイクルとして35サイクル繰り返しさらに72 $^{\circ}$ Cで5分間放置しPCR反応を行った。

このPCR反応物を電気泳動し、285~296塩基付近に増幅されるバンドが観察されたPAC DNA poolについてののみ同様の方法でさらに2度PCRを行いクローンの絞り込みを行った。

その結果を図3に示す。dJ142A6、dJ332G17、dJ345P21、dJ369D13の4つのPACクローンにおいて、285~296塩基付

近に増幅されるバンドが観察された。図3にはd J 1 4 2 A 6、d J 3 3 2 G 1
7、d J 3 6 9 D 1 3のみの結果を示す。

同様にして、1 p 3 6 . 2 3 - 3 1 領域の欠失を確認した他のプライマーセッ
ト S H G C - 3 1 4 5 3、S G C - 3 0 3 4 3、D 1 S 2 0 2 1、D 1 S 1 7 6
5 8、P G D、D 1 S 2 7 3 6によるスクリーニングを行った結果、S H G C - 3
1 4 5 3を除く5つのプライマーセットで増幅が観察されたものが14クローン
単離された。

以上により得られたクローンを1 s t スクリーニングとした。1 s t スクリー
ニングにより得られた18クローンを表3に示す。

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表 3

1stスクリーニングにより得られた18個のPACクローンとそのコンティグ
 太字は複数のプライマーにより検出されたクローン

プライマーセット	得られたPACクローン	コンティグ
SGC-30343	dJ99E23, dJ371E1, dJ310M20	1
D1S2021	dJ326J10, dJ694C14, dJ736L20, dJ739D18, dJ739F17, dJ756A17	2
D1S1768	dJ266H16, dJ326J10, dJ373D24	
PGD	dJ92K14, dJ266H16, dJ373D24, dJ585H7, dJ686A20	
D1S244	dJ142A6, dJ332G17, dJ345P21, dJ369D13	3
D1S2736	dJ142A6, dJ345P21, dJ369D13	

前記 1 s t スクリーニングにより得られた合計 18 クローンは、NB-1 及び MASS-NB-SCH-1 の 2 株において欠失した領域のゲノムシーケンスを保持していると考えられ、複数のプライマーセットにより検出されるものがあることから、おおまかなコンティグを 3 つ形成した（それぞれをコンティグ 1、
5 コンティグ 2、コンティグ 3 とする）。1 s t スクリーニングによって得られた 18 の PAC クローンのコンティグを図 4 に示す。

コンティグ 1 から dJ99E23、dJ371E1 の 2 クローン、コンティグ 2 から dJ326J10、dJ739D18、dJ756A17、dJ373D24 の 4 クローン、コンティグ 3 から dJ369D13、dJ142A6、dJ
10 345P21 の 3 クローン、合計 9 クローンの PAC について両末端の塩基配列を RPN2434（キット：アマシャム社製）を用いて以下の方法により決定した。2 μ g の PAC DNA、6 pmol の SP6 または T7 プライマー、2 μ l の A、C、G、T の各試薬の混合液（キット付属品）に滅菌蒸留水を加えてトータル 6 μ l の混合液を調製する。この混合液を 95 $^{\circ}$ C で 2 分間変性させた後、
15 95 $^{\circ}$ C で 30 秒間、50 $^{\circ}$ C で 30 秒間、72 $^{\circ}$ C で 1 分間を 1 サイクルとして 30 サイクル繰り返し PCR 反応を行った。反応液に 5 μ l の反応停止液（キット付属品）を加え、さらに 95 $^{\circ}$ C で 2 分間変性させ氷冷し、6 % のアクリルアミドゲルで電気泳動を行い、シーケンスした。得られた塩基配列情報から新たにウォーキングプライマーを 10 セット設計した。

20 表 4 に選抜したクローンとそれぞれのプライマーセットのシーケンスを示す。

表 4

2nd、3rdスクリーニングで使用了したプライマーセットのシーケンス

コンティグ	PACクローン	プライマーセット	シーケンス(F) 5'-3'	(配列番号)	シーケンス(R) 5'-3'	(配列番号)
1	dJ99E23	dJ99E23-T7	GCGTTAGGAATTGAACAG	(29)	TGGAGTATGTCTGCCGG	(37)
	dJ371E1	dJ371E1-SP6	CTGCTTCGGAGGTCAGCC	(21)	GTGACTGCGTGATTGCAT	(31)
2	dJ326J10	dJ326J10-SP6	ACTTGGAGGATTTATTG	(6)	ATACATAAGATAAAGAATC	(11)
	dJ739D18	dJ739D18-SP6	TAGCTGAAGCAATCTATT	(34)	TTTTCCGCTTTCCACAC	(41)
	dJ756A17	dJ756A17-SP6	AGTATGCTGTGTGCCAG	(9)	GAGGACTAGTGTGAGAAA	(27)
	dJ373D24	dJ373D24-SP6	AGTAGACACGGGTATCAG	(8)	CCACCTCCAAATCCTGAC	(16)
3	dJ369D13	dJ369D13-T7	CGGCTTAAATCTGATA	(19)	CTCAATACTGGATCTGTG	(20)
	dJ142A6	dJ142A6-SP6	AGTATGGCAGACTTGATG	(10)	ATGAATGGGACAAACTTC	(14)
		dJ142A6-T7	CTGGGCAAGCTAATTTTG	(23)	TCTGGACGTACTCIGTTC	(35)
	dJ345P21	dJ345P21-T7	CCCTCACCCACACTTCAC	(17)	TTTCCTGCCTGTGGCCTC	(40)

コンティグ	PACクローン	プライマーセット	シーケンス(F) 5'-3'	(配列番号)	シーケンス(R) 5'-3'	(配列番号)
1	dJ587C9	dJ587C9-T7	ATATTGCAGACTGAACAG	(12)	AAGCAGTTTTCCCATAA	(3)

新たに設計したプライマーセットを用いて再度PAC DNA poolからPCRによるPACクローンを選抜し、同様に、選抜されたクローンの両末端シーケンスによるウォーキングプライマーの設計、ウォーキングによるコンティグの決定のプロセスを繰り返した。これを2nd、3rdスクリーニングとした。

5 表5に選抜したクローンとそれぞれのプライマーセットのシーケンスを示す。

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表 5

2nd、3rdスクリーニングにより得られたPACクローン
太字は複数のプライマーにより検出されたクローン

2nd		新たに得られたPACクローン	コンティグ
プライマーセット			
dJ99E23-T7	dJ694C14		1
dJ371E1-SP6	dJ587C9、dJ677L1、dJ758J12		
dJ326J10-SP6	dJ204G8、dJ519P22、dJ548K20、dJ668B3、dJ1127L21、dJ694C14		2
dJ739D18-SP6	-		
dJ756A17-SP6	dJ519P22、dJ668B3、dJ1127L21		
dJ373D24-SP6	dJ332G17、dJ345P21、dJ603E7、dJ636D16、dJ585H7、dJ686A20		
dJ369D13-T7	dJ529H5、dJ534G20、dJ625A13、dJ734H19		3
dJ142A6-SP6	-		
dJ142A6-T7	dJ534G20		
dJ345P21-T7	dJ603E7、dJ636D16		
3rd		新たに得られたPACクローン	コンティグ
プライマーセット			
dJ587C9-T7	dJ1028O13、dJ1028N11		1

2nd、3rdスクリーニングに用いたウォーキングプライマーのシーケンスを表4に示す。最終的に34クローンのPACからなる1つのコンティグが得られた。得られた結果を図5に示す。

(実施例6) NB-1及びMASS-NB-SCH-1株の1p36における

5 ゲノム欠失部位の特定

実施例4により、NB-1及びMASS-NB-SCH-1は1p36の少なくともSGC-30343からD1S2736で規定される領域に両染色体の欠失を有するものと判断したが、欠失領域の両末端を特定するために以下の操作を行った。

10 実施例5により得られたPACクローンによるコンティグ情報から欠失領域付近に新たにプライマーを合成し、NB-1及びMASS-NB-SCH-1株と陽性コントロールのPCRを以下の条件で行い、特異的バンドの増幅の有無を調べた。LA PCR kit (宝酒造社製)を用いて以下の方法でPCRを行った。

15 1 μ lのゲノムDNA溶液(100ng/ μ l)、1 μ lの10xLA PCR buffer、0.6 μ lのdNTPs(2.5mM:キット付属品)、0.1 μ lのプライマーF(100mM)、0.1 μ lのプライマーR(100mM)、7.15 μ lの滅菌蒸留水、および0.05 μ lのLA Taq(5u/ μ l:キット付属品)を混合し10 μ lの混合液を調製した。混合液を95℃で
20 2分間変性させた後、95℃で30秒間、58℃で1分間を1サイクルとして30サイクル繰り返しPCR反応を行った。

このPCR反応物を2.5%のアガロースゲルを用いて電気泳動した。得られた結果を図6に示す。

これらの結果により、図7に示すようなPACクローンであるdJ10280
25 13のSP6側プライマーセットとPACクローンであるdJ371E1のSP6側プライマーセットとの間からプライマーセットD1S2736とPACクロ

ーンである d J 1 4 2 A 6 の T 7 側プライマーセットとの間で規定される 1 p 3 6 におけるゲノム欠失領域の範囲が得られた。

この欠失領域を全て網羅する最小限の P A C クローン群を図 8 に示す。これらの P A C クローンをシーケンスすることで N B - 1、M A S S - N B - S C H
5 - 1 の欠失領域中に含まれる塩基配列が決定される。

なお、表 6 は本発明により明らかとなった 1 p 3 6 におけるゲノム欠失領域範囲を決定したプライマーセットのシーケンスを示す表である。

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表 6

D1S2736、dJ1028O13-SP6、dJ371E1-SP6、dJ142A6-T7のシーケンス

プライマーセット	シーケンス(F) 5'-3' (配列番号)	シーケンス(R) 5'-3' (配列番号)
D1S2736	CCTCCAGGGTATTCTTGG (18)	TTTTGAGGTGTGAGAGCAG (43)
dJ1028O13-SP6	GTGTTCCATCATATGGTCA (36)	TGGTTTAGAGGCTTTTGC (33)
dJ371E1-SP6	CTGCTTCGGAGGTCAGCC (22)	GTGACTGCGTGATTGCAT (31)
dJ142A6-T7	CTGGGCAAGCTAATTTTG (24)	TCGGACGTACTCTGTTT (35)

(実施例7) 1p36欠失領域の塩基配列情報

欠失領域の塩基配列情報を得る目的で、インターネットの遺伝子マップデータベース（3万個以上の染色体別にマッピングされた遺伝子、EST等のヒトゲノム情報が登録されているデータベース：<http://www.ncbi.nlm.nih.gov/genemap99/>）を、先の実施例で用いたプライマーD1S244等をキーワードとして検索すると、数々の遺伝子およびESTがヒットした。また、ゲノムシーケンスの一部についてインターネット [NCBIのBLAST (National Center of Biotechnology Information USA, <http://www.ncbi.nlm.nih.gov/BLAST>) を介したDNA配列のホモロジー検索を行った結果、ESTの一部が判明した。これらの配列に関する情報を表7に示し、塩基配列が登録されているものについては、配列表に記載した。

表 7

表 7: 欠失領域の塩基配列情報

塩基配列 番号	名称または登録番号	定義および特徴	配列長(bp)	配列番号
1	UFD2(AF043117)	ubiquitin-fusion degradation protein 2	3909	44
2	KIF1B	kinesin-like protein KIF1B		
3	CORT(NM001302)	preprocortistatin		
4	PEX-14(AB017546)	ペルオキシソーム膜タンパク	318	45
5	KIAA0684(AB014584)	Homo sapiens clone 686 protein (KIAA0684) mRNA.		46
6	KIAA0591(AB011163)	Homo sapiens mRNA for KIAA0591 protein, partial cds.	5312	47
7	AI080353(EST)	ox80f04.s1 Soares NhHMPu S1 Homo sapiens cDNA clone	5368	48
8	D79099(EST)	HUM531D06B Human placenta polyA+ (TFujiwara) Homo sapiens cDNA clone	422	49
9	AA059029(EST)	zf63h02.s1 Soares retina N2b4HR Homo sapiens cDNA clone	320	50
10	AA628589(EST)	af39g05.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone	446	51
11	AA728950(EST)	nw02c08.s1 NCI CGAP Pr16 Homo sapiens cDNA clone	443	52
12	AA328945(EST)	EST32514 Embryo, 12 week I Homo sapiens cDNA clone	437	53
13	R89689(EST)	ym99c01.r1 Soares adult brain N2b4HB55Y Homo sapiens cDNA clone	302	54
14	AA018367(EST)	ze41e05.r1 Soares retina N2b4HR Homo sapiens cDNA clone	450	55
15	Z19845(EST)	HSAAAAUKT H, Human adult Brain Cortex tissue Homo sapiens cDNA	504	56
16	AI050021(EST)	an22d04.x1 Gessler Wilms tumor Homo sapiens cDNA clone	281	57
17	R67760(EST)	yi28f03.r1 Soares placenta Nb2HP Homo sapiens cDNA clone	434	58
			496	59

(実施例8) 1 p 3 6 欠失領域のシーケンシング

実施例6と同様に、欠失領域をほぼ全て網羅するPACクローン群を選択した。これらのPACクローンは、dJ371E1、dJ756A17、dJ266I16、およびdJ345P21であり、それらを全体とした構成図を図9に示す。

5 従って、前記PACクローンの各々をシーケンスして、繋ぎあわせれば欠失領域の塩基配列のほぼ全長が決定できる。

そこで、各クローンのシーケンシングをネステッドディレーション (ND) 法により決定した。服部正平、蛋白質 核酸 酵素 42, 17, 296-303 (1997)、またはHattori M.等, Nucl. Acids Res. 25, 1802-1808 (1997) を参照。

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以上のようにして、欠損領域を含むが、各クローンの部分配列を決定できた。すなわち、PACクローンdJ371E1については、欠損領域を含むが連続する5つの部分配列の塩基配列が解明された。これらの部分配列は、dJ371E1-1 (配列番号60)、dJ371E1-2 (配列番号61)、dJ371E1-3 (配列番号62)、dJ371E1-4 (配列番号63)、dJ371E1-5 (配列番号64) と命名された。それぞれの塩基配列を配列表に記載した。また、PACクローンdJ756A17についても、欠損領域を含むが連続する7つの部分配列の塩基配列が解明された。これらの部分配列は、dJ756A17-1 (配列番号65)、dJ756A17-2 (配列番号66)、dJ756A17-3 (配列番号67)、dJ756A17-4 (配列番号68)、dJ756A17-5 (配列番号69)、dJ756A17-6 (配列番号70)、およびdJ756A17-7 (配列番号71) と命名された。それぞれの塩基配列を配列表に記載した。また、PACクローンdJ266I16についても、欠損領域を含むが連続する9つの部分配列の塩基配列が解明された。これらの部分配列は、dJ266I16-1 (配列番号72)、dJ266I16-2 (配列番号73)、dJ266I16-3 (配列番号74)、dJ266I16-4 (配列番号75)、

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d J 2 6 6 I 1 6 - 5 (配列番号 7 6)、d J 2 6 6 I 1 6 - 6 (配列番号 7 7)、
d J 2 6 6 I 1 6 - 7 (配列番号 7 8)、d J 2 6 6 I 1 6 - 8 (配列番号 7 9)、
および d J 2 6 6 I 1 6 - 9 (配列番号 8 0) と命名された。それぞれの塩基配
列を配列表に記載した。また、PACクローン d J 3 4 5 p - 2 1 についても、
5 欠損領域を含むが連続する 8 つの部分配列の塩基配列が解明された。これらの部
分配列は、d J 3 4 5 p - 2 1 - 1 (配列番号 8 1)、d J 3 4 5 p - 2 1 - 2 (配
列番号 8 2)、d J 3 4 5 p - 2 1 - 3 (配列番号 8 3)、d J 3 4 5 p - 2 1 -
4 (配列番号 8 4)、d J 3 4 5 p - 2 1 - 5 (配列番号 8 5)、d J 3 4 5 p -
2 1 - 6 (配列番号 8 6)、d J 3 4 5 p - 2 1 - 7 (配列番号 8 7)、および d
10 J 3 4 5 p - 2 1 - 8 (配列番号 8 8) と命名された。それぞれの塩基配列を配
列表に記載した。

(実施例 9) 欠失領域に含まれる塩基配列に基づく、ヒト神経芽細胞腫の予後
に関する診断

小児神経芽細胞腫の患者の細胞を用い、今回明らかになった 1 p 3 6 における
15 L O H の有無と癌の予後に関する種々のファクターとの相関を検討した。その結
果を表 8 に示す。

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表 8

1p36のLOHを用いたヒト神経芽細胞腫の予後の同定

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(a) 患者の年齢との相関 : $p=0.008$

	LOH有り	LOH無し	計
1歳未満	23	103	126
1歳以上	27	51	78
計	50	154	204

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(b) 原発巣との相関 : $p=0.000$

	LOH有り	LOH無し	計
副腎	42	81	123
副腎以外	8	78	85
計	50	159	209

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(c) trkA発現レベルとの相関 : $p=0.003$

	LOH有り	LOH無し	計
trkA高発現	24	36	60
trkA低発現	18	79	97
計	42	115	157

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(d) N-mycの有無との相関 : $p=0.000$

	LOH有り	LOH無し	計
N-myc有り	17	3	180
N-myc無し	31	149	20
計	48	152	200

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一般的に癌遺伝子である *trkA* が高発現している患者は予後が不良とされている。Nakagawara A. 等 *Cancer Res.* 52, 1364-1368 (1992), Nakagawara A. 等 *N. Engl. J. Med.* 328, 847-854 (1993) を参照。

- 5 実際、小児神経芽細胞腫の患者157症例で1p36におけるLOHの有無を *trk* 遺伝子の発現の強度と比較して調べたところ、1p36にLOHが存在する患者42症例中、*trk* 高発現の患者が24例、低発現の患者は18例となった。逆に1p36にLOHが存在しない患者では115例中、*trk* 高発現の患者が36症例、低発現の患者は79例となった。1p36におけるLOHの有無と *trk* 発現の間に良い相関が得られた。
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- 同様に、発症年齢が高い患者 (Nakagawara A. 等 *Med. Pediatr. Oncol.* 31, 113-115 (1998))、原発巣が副腎である患者 (Nakagawara A. 等 *Surgery* 104, 34-40 (1988))、及び癌遺伝子である *N-myc* が高発現している患者も (Brodeur GM. 等 *Science* 224, 1121-1124 (1984)) も一般的に予後不良であるといわれているが、1p36におけるLOHの有無との相関が非常に安定して得られた。
- 15

以上のことから1p36におけるLOHの有無が予後と同定する優れたマーカーであることが明らかになった。

20 産業上の利用可能性

- 本発明に従い、ヒト神経芽細胞腫の第1番染色体短腕36位に共通に存在するホモ接合性欠失領域を同定した。得られた欠失領域の塩基配列の情報に基づき、腫瘍の診断、予後同定の方法およびそのために使用可能な腫瘍マーカーを設計することが可能となり、さらに、該領域の塩基配列に基づき、腫瘍の遺伝子診断方法、抗癌剤の開発、腫瘍病体メカニズムの解析方法、腫瘍病体メカニズムの解析に用いる試薬の開発も可能となる。
- 25

請求の範囲

1. ヒト神経芽細胞腫の第1番染色体短腕36位(1p36)に共通に存在するホモ接合性欠失(Homozygous Deletion)領域。
2. 少なくともPACクローンであるdJ1028013のSP6側プライマーセットとPACクローンであるdJ371E1のSP6側プライマーセットとの間から、プライマーセットD1S2736とPACクローンであるdJ142A6のT7側プライマーセットとの間で、規定されることを特徴とする請求項1に記載の領域。
3. 前記領域が、さらに少なくともプライマーセットSGC30343およびD1S2736で規定されることを特徴とする、請求項1または2に記載の領域。
4. ヒト神経芽細胞腫の細胞株NB-1及びMASS-NB-SCH-1株において欠失していることを特徴とする、請求項1または2に記載の領域。
5. ヒト神経芽細胞腫の細胞株NB-1及びMASS-NB-SCH-1株において欠失していることを特徴とする、請求項3に記載の領域。

pat

PATENT COOPERATION T A T Y

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PCT/JP00/05930

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

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To:

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US Department of Commerce
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2011 South Clark Place Room
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Date of mailing: 08 March 2001 (08.03.01)	
International application No.: PCT/JP00/05930	Applicant's or agent's file reference: FP00-0197-00
International filing date: 31 August 2000 (31.08.00)	Priority date: 31 August 1999 (31.08.99)
Applicant: NAKAGAWARA, Akira	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International preliminary Examining Authority on:
19 January 2001 (19.01.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer: J. Zahra Telephone No.: (41-22) 338.83.38
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3869984

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

HASEGAWA, Yoshiki
Soei Patent and Law Firm, Okura-
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JAPON

Date of mailing (day/month/year) 01 May 2001 (01.05.01)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference FP00-0197-00	
International application No. PCT/JP00/05930	International filing date (day/month/year) 31 August 2000 (31.08.00)

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

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3. Further observations, if necessary:

4. A copy of this notification has been sent to:

<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input checked="" type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Yukari NAKAMURA

Telephone No.: (41-22) 338.83.38

A. 発明の属する分野の分類 (国際特許分類 (IPC))

Int. Cl⁷ C12N15/09

B. 調査を行った分野

調査を行った最小限資料 (国際特許分類 (IPC))

Int. Cl⁷ C12N15/00-15/90

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用した電子データベース (データベースの名称、調査に使用した用語)

BIOSIS/MEDLINE/WPI (STN)

C. 関連すると認められる文献

引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する 請求の範囲の番号
A	GENOMICS, 49, May 1998 Vincent J. Kidd et al., "Duplication of the DR3 gene on human chromosome 1p36 and its deletion in human neuroblastoma", p. 385-393	1-5
A	Proc. Natl. Acad. Sci. USA, 92(12), Jun. 1995 Peter S. White et al., "A region of consistent deletion in neuroblastoma maps within human chromosome 1p36.2-36.3", p. 5520-5524	1-5

☒ C欄の続きにも文献が列举されている。

☐ パテントファミリーに関する別紙を参照。

* 引用文献のカテゴリー

- 「A」 特に関連のある文献ではなく、一般的技術水準を示すもの
 「E」 国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
 「L」 優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献 (理由を付す)
 「O」 口頭による開示、使用、展示等に言及する文献
 「P」 国際出願日前で、かつ優先権の主張の基礎となる出願

- の日の後に公表された文献
 「T」 国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
 「X」 特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
 「Y」 特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
 「&」 同一パテントファミリー文献

国際調査を完了した日

22.11.00

国際調査報告の発送日

05.12.00

国際調査機関の名称及びあて先

日本国特許庁 (ISA/J P)

郵便番号 100-8915

東京都千代田区霞が関三丁目4番3号

特許庁審査官 (権限のある職員)

鈴木 恵理子

4B

9838

電話番号 03-3581-1101 内線 3448

国際調査報告

(法8条、法施行規則第40、41条)
[PCT18条、PCT規則43、44]

出願人又は代理人 の書類記号	F P 0 0 - 0 1 9 7 - 0 0	今後の手続きについては、国際調査報告の送付通知様式(PCT/ISA/220)及び下記5を参照すること。	
国際出願番号 PCT/J P 0 0 / 0 5 9 3 0	国際出願日 (日.月.年) 3 1 . 0 8 . 0 0	優先日 (日.月.年) 3 1 . 0 8 . 9 9	
出願人(氏名又は名称) 久光製薬株式会社			

国際調査機関が作成したこの国際調査報告を法施行規則第41条(PCT18条)の規定に従い出願人に送付する。
この写しは国際事務局にも送付される。

この国際調査報告は、全部で 3 ページである。

☐ この調査報告に引用された先行技術文献の写しも添付されている。

1. 国際調査報告の基礎

a. 言語は、下記に示す場合を除くほか、この国際出願がされたものに基づき国際調査を行った。

☐ この国際調査機関に提出された国際出願の翻訳文に基づき国際調査を行った。

b. この国際出願は、ヌクレオチド又はアミノ酸配列を含んでおり、次の配列表に基づき国際調査を行った。

☐ この国際出願に含まれる書面による配列表

☐ この国際出願と共に提出されたフレキシブルディスクによる配列表

☐ 出願後に、この国際調査機関に提出された書面による配列表

☐ 出願後に、この国際調査機関に提出されたフレキシブルディスクによる配列表

☐ 出願後に提出した書面による配列表が出願時における国際出願の開示の範囲を超える事項を含まない旨の陳述書の提出があった。

☐ 書面による配列表に記載した配列とフレキシブルディスクによる配列表に記載した配列が同一である旨の陳述書の提出があった。

2. ☐ 請求の範囲の一部の調査ができない(第I欄参照)。

3. ☐ 発明の単一性が欠如している(第II欄参照)。

4. 発明の名称は ☒ 出願人が提出したものを承認する。

☐ 次に示すように国際調査機関が作成した。

5. 要約は ☒ 出願人が提出したものを承認する。

☐ 第III欄に示されているように、法施行規則第47条(PCT規則38.2(b))の規定により国際調査機関が作成した。出願人は、この国際調査報告の発送の日から1カ月以内にこの国際調査機関に意見を提出することができる。

6. 要約書とともに公表される図は、

第 8 図とする。 ☒ 出願人が示したとおりである。

☐ なし

☐ 出願人は図を示さなかった。

☐ 本図は発明の特徴を一層よく表している。

C (続き) . 関連すると認められる文献		
引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する 請求の範囲の番号
A	Oncogene, 8(12), Dec. 1993 Barker PE et al., "Translocation junctions cluster at the distal short arm of chromosome 1(1p36.1-2) in human neuroblastoma cells.", p. 3353-3358	1 - 5
A	Cancer Lett, 130(1-2), Aug. 1998 Iolascon A. et al., "Interstitial and large chromosome 1p deletion occurs in localized and disseminated neuroblastomas and predicts an unfavourable outcome", p. 83-92	1 - 5
T	Oncogene, 19(37), Aug. 31 2000, p. 4302-4307 Nakagawara A et al., "Identification and characterization of a 500-kb homozygously deleted region at 1p36.2-36.3 in a neuroblastoma cell line", p. 4302-4307	1 - 5



DOCUMENTS CONSIDERED TO BE RELEVANT															
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.CI.7)												
① X ✓	WHITE P S ET AL: "Molecular analysis of the region of distal 1p commonly deleted in Neuroblastoma" EUROPEAN JOURNAL OF CANCER, PERGAMON PRESS, OXFORD, GB, vol. 33, no. 12, October 1997 (1997-10), pages 1957-1961, XP004284624 ISSN: 0959-8049 * abstract * * page 1959, left-hand column, paragraph 4 - page 1960, right-hand column, paragraph 1; figures 1,2 *	1-5	C12N15/09												
② X ✓	HIRAIWA HISAYUKI ET AL: "A neuroblastoma cell line derived from a case detected through a mass screening system in Japan: A case report including the biological and phenotypic characteristics of cell line." CANCER, vol. 79, no. 10, 1997, pages 2036-2044, XP002218785 ISSN: 0008-543X * abstract * * page 2039, right-hand column, paragraph 2 - page 2043, right-hand column; figure 9 *	1-5	TECHNICAL FIELDS SEARCHED (Int.CI.7) C12N C07K												
③ X ✓	SCHWAB MANFRED ET AL: "Genomic instability in 1p and human malignancies." GENES CHROMOSOMES & CANCER, vol. 16, no. 4, 1996, pages 211-229, XP001118678 ISSN: 1045-2257 * page 212, right-hand column - page 213, left-hand column, paragraph 1; figure 1 * --- -/--	1-5													
4	The supplementary search report has been based on the last set of claims valid and available at the start of the search.														
Place of search MUNICH		Date of completion of the search 30 October 2002	Examiner Strobel, A												
<table border="0"><tr><td>CATEGORY OF CITED DOCUMENTS</td><td>T : theory or principle underlying the invention</td></tr><tr><td>X : particularly relevant if taken alone</td><td>E : earlier patent document, but published on, or after the filing date</td></tr><tr><td>Y : particularly relevant if combined with another document of the same category</td><td>D : document cited in the application</td></tr><tr><td>A : technological background</td><td>L : document cited for other reasons</td></tr><tr><td>O : non-written disclosure</td><td></td></tr><tr><td>P : intermediate document</td><td>& : member of the same patent family, corresponding document</td></tr></table>				CATEGORY OF CITED DOCUMENTS	T : theory or principle underlying the invention	X : particularly relevant if taken alone	E : earlier patent document, but published on, or after the filing date	Y : particularly relevant if combined with another document of the same category	D : document cited in the application	A : technological background	L : document cited for other reasons	O : non-written disclosure		P : intermediate document	& : member of the same patent family, corresponding document
CATEGORY OF CITED DOCUMENTS	T : theory or principle underlying the invention														
X : particularly relevant if taken alone	E : earlier patent document, but published on, or after the filing date														
Y : particularly relevant if combined with another document of the same category	D : document cited in the application														
A : technological background	L : document cited for other reasons														
O : non-written disclosure															
P : intermediate document	& : member of the same patent family, corresponding document														



DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	WHITE P S ET AL: "Physical mapping of the CA6, EN01, and SLC2A5 (GLUT5) genes and reassignment of SLC2A5 to 1p36.2." CYTOGENETICS AND CELL GENETICS, vol. 81, no. 1, 1998, pages 60-64, XP002218787 ISSN: 0301-0171 * page 60, right-hand column - page 62, right-hand column, paragraph 2; figures 1,2; table 1 *	1-5	
P,X	SPIEKER NICOLE ET AL: "An integrated 5-Mb physical, genetic, and radiation hybrid map of a 1p36.1 region implicated in neuroblastoma pathogenesis." GENES CHROMOSOMES & CANCER, vol. 27, no. 2, February 2000 (2000-02), pages 143-152, XP002218801 ISSN: 1045-2257 * abstract * * page 144, right-hand column, paragraph 4 * * page 145, right-hand column, paragraph 2; table 1 * * page 148, left-hand column, paragraph 2 - page 148, right-hand column, paragraph 1 * * page 151, left-hand column, paragraph 4 - paragraph 5 * --- -/--	1-5	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
The supplementary search report has been based on the last set of claims valid and available at the start of the search.			
Place of search MUNICH		Date of completion of the search 30 October 2002	Examiner Strobel, A
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

4
EPO FORM 1503 03 82 (P04C04)



European Patent
Office

SUPPLEMENTARY
EUROPEAN SEARCH REPORT

Application Number
EP 00 95 6853

DOCUMENTS CONSIDERED TO BE RELEVANT															
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)												
6 A ✓	IOANNOU P A ET AL: "A NEW BACTERIOPHAGE P1-DERIVED VECTOR FOR THE PROPAGATION OF LARGE HUMAN DNA FRAGMENTS" NATURE GENETICS, NEW YORK, NY, US, vol. 6, 1994, pages 84-89, XP000770742 ISSN: 1061-4036 * abstract * * page 85, left-hand column, paragraph 3 - page 86, right-hand column, paragraph 1; figures 1,2 * -----	1-5													
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)												
The supplementary search report has been based on the last set of claims valid and available at the start of the search.															
Place of search MUNICH		Date of completion of the search 30 October 2002	Examiner Strobel, A												
<table border="0"><tr><td>CATEGORY OF CITED DOCUMENTS</td><td>T : theory or principle underlying the invention</td></tr><tr><td>X : particularly relevant if taken alone</td><td>E : earlier patent document, but published on, or after the filing date</td></tr><tr><td>Y : particularly relevant if combined with another document of the same category</td><td>D : document cited in the application</td></tr><tr><td>A : technological background</td><td>L : document cited for other reasons</td></tr><tr><td>O : non-written disclosure</td><td>& : member of the same patent family, corresponding document</td></tr><tr><td>P : intermediate document</td><td></td></tr></table>				CATEGORY OF CITED DOCUMENTS	T : theory or principle underlying the invention	X : particularly relevant if taken alone	E : earlier patent document, but published on, or after the filing date	Y : particularly relevant if combined with another document of the same category	D : document cited in the application	A : technological background	L : document cited for other reasons	O : non-written disclosure	& : member of the same patent family, corresponding document	P : intermediate document	
CATEGORY OF CITED DOCUMENTS	T : theory or principle underlying the invention														
X : particularly relevant if taken alone	E : earlier patent document, but published on, or after the filing date														
Y : particularly relevant if combined with another document of the same category	D : document cited in the application														
A : technological background	L : document cited for other reasons														
O : non-written disclosure	& : member of the same patent family, corresponding document														
P : intermediate document															

4

EPO FORM 1503 03 82 (P04C04)

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

47
Translation

Applicant's or agent's file reference FP00-0197-00	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/JP00/05930	International filing date (day/month/year) 31 August 2000 (31.08.00)	Priority date (day/month/year) 31 August 1999 (31.08.99)
International Patent Classification (IPC) or national classification and IPC C12N 15/09		
Applicant HISAMITSU PHARMACEUTICAL CO., INC.		

<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of <u>4</u> sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of _____ sheets.</p>
<p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the report</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input checked="" type="checkbox"/> Certain observations on the international application</p>

Date of submission of the demand 19 January 2001 (19.01.01)	Date of completion of this report 20 August 2001 (20.08.2001)
Name and mailing address of the IPEA/JP	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/JP00/05930

I. Basis of the report

1. With regard to the **elements** of the international application:*

- ☒ the international application as originally filed
- ☐ the description:
 pages _____, as originally filed
 pages _____, filed with the demand
 pages _____, filed with the letter of _____
- ☐ the claims:
 pages _____, as originally filed
 pages _____, as amended (together with any statement under Article 19
 pages _____, filed with the demand
 pages _____, filed with the letter of _____
- ☐ the drawings:
 pages _____, as originally filed
 pages _____, filed with the demand
 pages _____, filed with the letter of _____
- ☐ the sequence listing part of the description:
 pages _____, as originally filed
 pages _____, filed with the demand
 pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item. These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/JP00/05930

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-5	YES
	Claims		NO
Inventive step (IS)	Claims	1-5	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-5	YES
	Claims		NO

2. Citations and explanations

Document 1: Vincent J. Kidd et al., "Duplication of the DR3 gene on human chromosome 1 p36 and its deletion in human neuroblastoma," Genomics, Vol. 49, May 1998, pp. 385-393

Claims 1-5

Document 1 describes a deletion at chromosome 1 p36 in a neuroblastoma, but this examination finds that a homozygous deleted region on chromosome 1 p36 shared by all neuroblastomas is not sufficiently identified, and this matter is not obvious from the description in this document. Therefore, the invention set forth in these Claims appears to involve an inventive step.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/JP00/05930

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 1

In the Specification only the deleted region in a specific neuroblastoma is disclosed, and this examination finds that in light of the other descriptions in the Specification and technical knowledge, this specific region is not sufficient to induce a blastoma. Therefore, the invention described in the above Claim is not sufficiently supported by the Specification.

Claims 2-5

This examination finds that in light of the description in the Specification and technical knowledge, the specific deleted region set forth in the above Claims is not a region sufficient to bring about a blastoma, and this being the case, this specific deleted region cannot be used as a prognostic marker for cancer. (Its use as a marker in the Examples merely suggests that a portion of this region exists that can serve as a marker provided the specific cancer-suppressing gene is not identified.) Therefore, the usefulness of the inventions set forth in these Claims is not disclosed in the Specification.

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

HASEGAWA, Yoshiki
Soei Patent and Law Firm, Okura-
honkan
6-12, Ginza 2-chome
Chuo-ku, Tokyo 104-0061
JAPON

RECEIVED

'00.11.13

Date of mailing (day/month/year) 03 November 2000 (03.11.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference FP00-0197-00	
International application No. PCT/JP00/05930	International filing date (day/month/year) 31 August 2000 (31.08.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 31 August 1999 (31.08.99)
Applicant HISAMITSU PHARMACEUTICAL CO., INC. et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
31 Augu 1999 (31.08.99)	11/245962	JP	20 Octo 2000 (20.10.00)
09 May 2000 (09.05.00)	2000/136266	JP	20 Octo 2000 (20.10.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

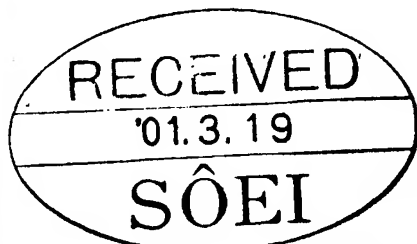
Facsimile No. (41-22) 740.14.35

Authorized officer

Magda BOUACHA

Telephone No. (41-22) 338.83.38

3.7



PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

HASEGAWA, Yoshiki
Soei Patent and Law Firm, Okura-
honkan
6-12, Ginza 2-chome
Chuo-ku, Tokyo 104-0061
JAPON

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 08 March 2001 (08.03.01)		IMPORTANT NOTICE	
Applicant's or agent's file reference FP00-0197-00			
International application No. PCT/JP00/05930	International filing date (day/month/year) 31 August 2000 (31.08.00)	Priority date (day/month/year) 31 August 1999 (31.08.99)	
Applicant HISAMITSU PHARMACEUTICAL CO., INC. et al			

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
AU, KR, US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

CA, EP, JP

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
08 March 2001 (08.03.01) under No. WO 01/16311

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a **demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

<p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No. (41-22) 740.14.35</p>	<p>Authorized officer J. Zahra</p> <p>Telephone No. (41-22) 338.83.38</p>
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TX 31651 epo nl
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Europäisches
Patentamt

Zweigstelle
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Patent Office

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The Hague
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des brevets

Département a
La Haye
Division de la
recherche

Grünecker, Kinkeldey,
Stockmair & Schwanhäusser
Anwaltssozietät
Maximilianstrasse 58
80538 München
ALLEMAGNE

Datum/Date

13.11.02

Zeichen/Ref./Réf. ✓ EP23820-031/gr	Anmeldung Nr./Application No./Demande n°/Patent Nr./Patent No./Brevet n°. 00956853.6-2401-JP0005930
Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire HISAMITSU PHARMACEUTICAL CO. INC., et al	

COMMUNICATION

The European Patent Office herewith transmits as an enclosure the European search report for the above-mentioned European patent application.

If applicable, copies of the documents cited in the European search report are attached.

☒ Additional set(s) of copies of the documents cited in the European search report is (are) enclosed as well.

REFUND OF THE SEARCH FEE

If applicable under Article 10 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.



図1

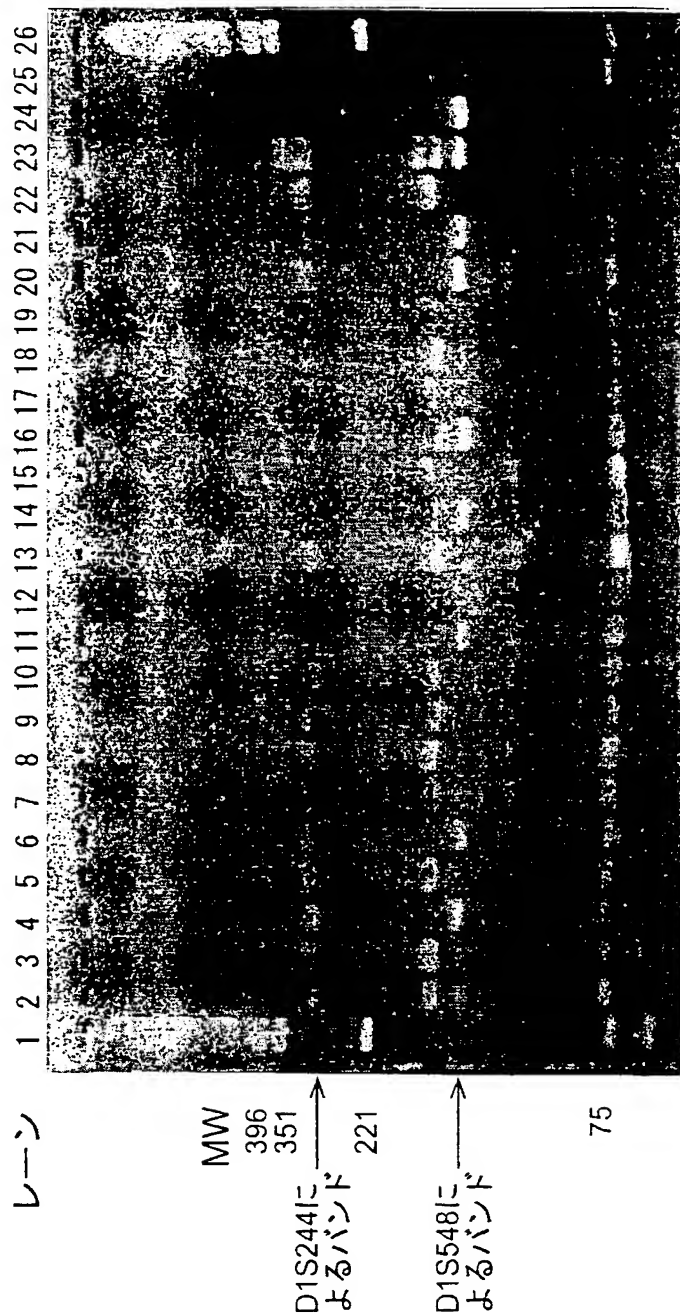


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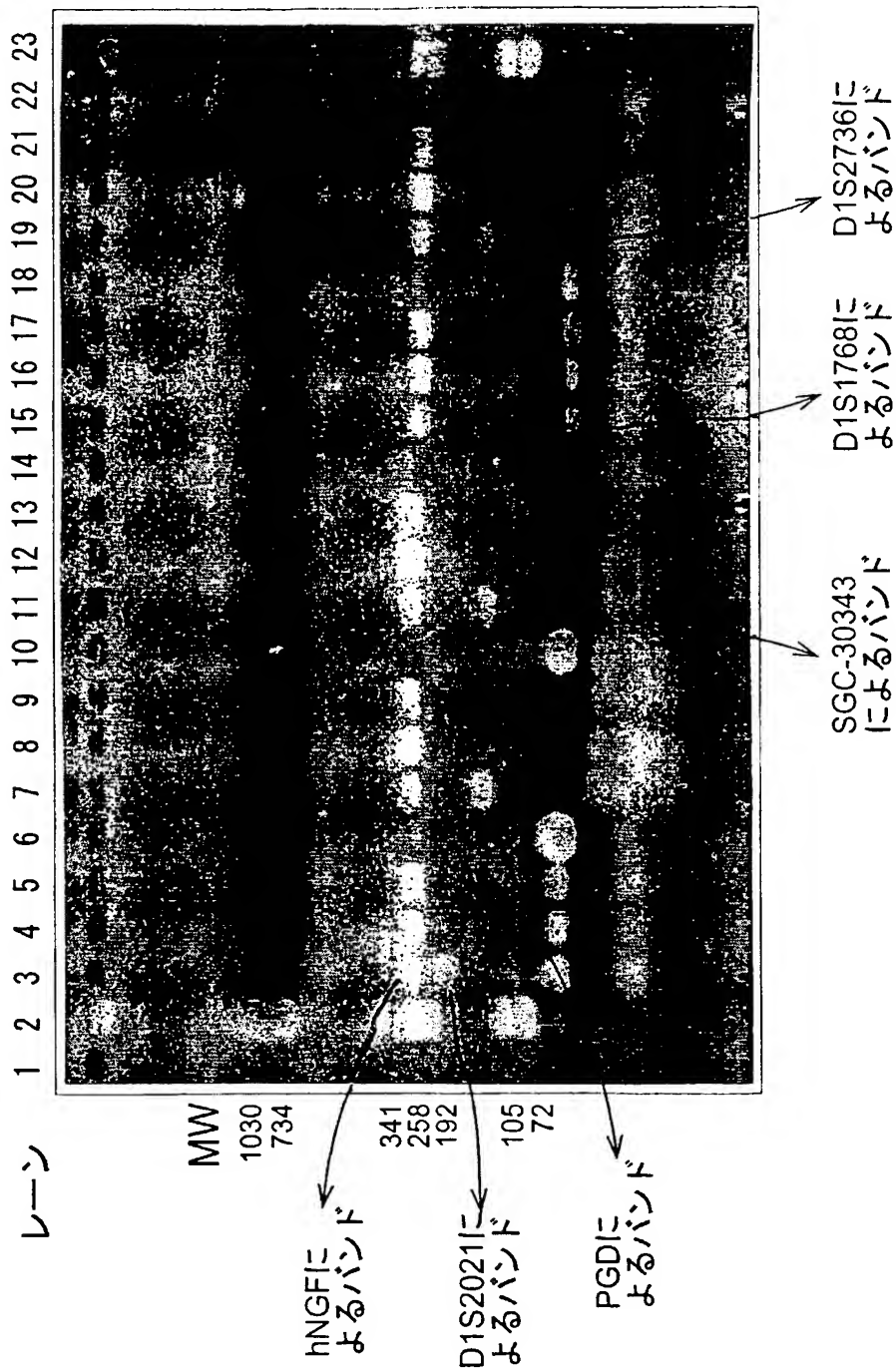


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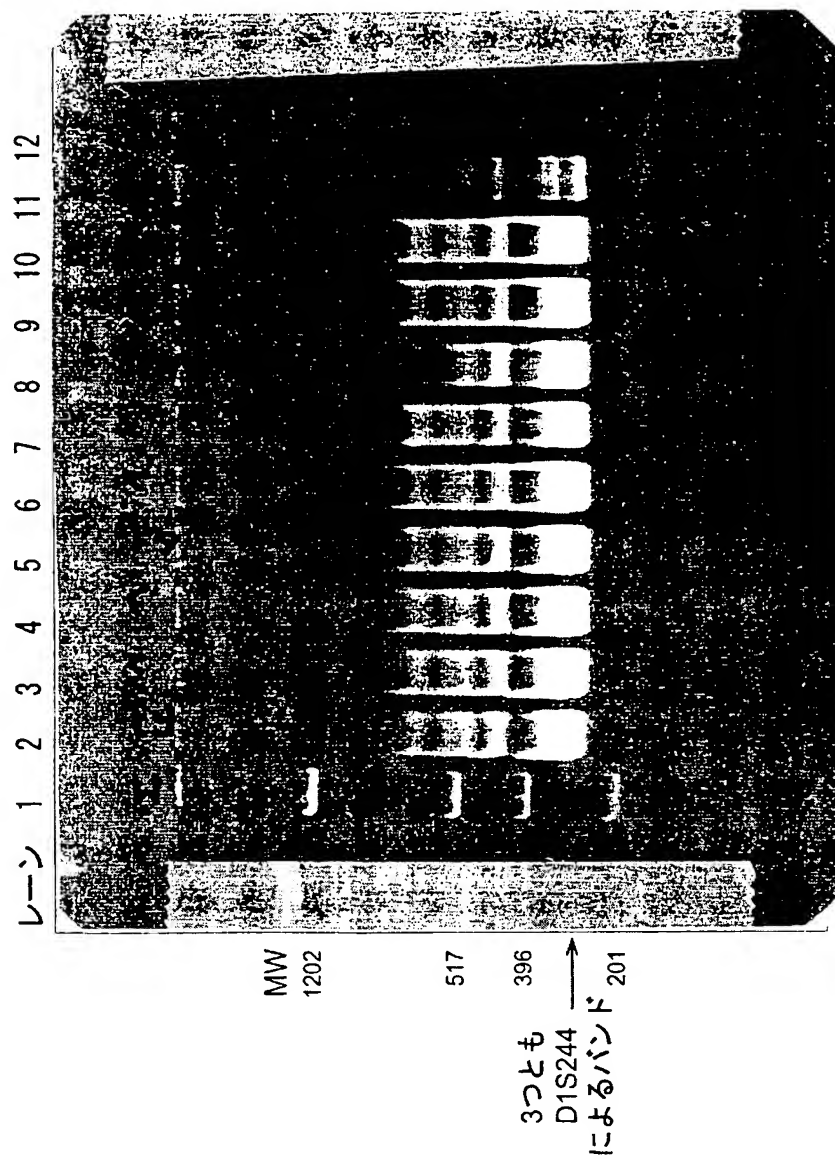


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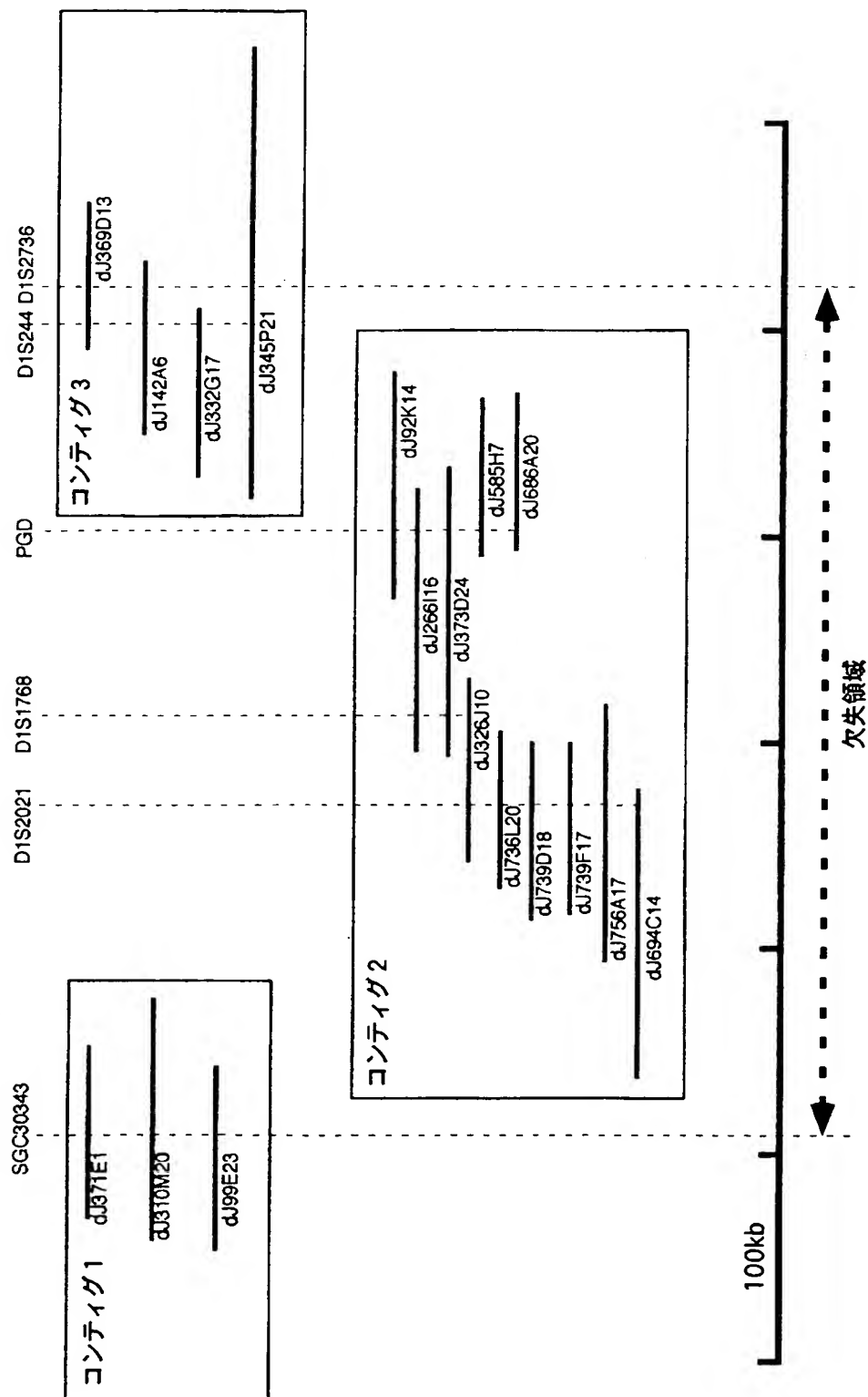


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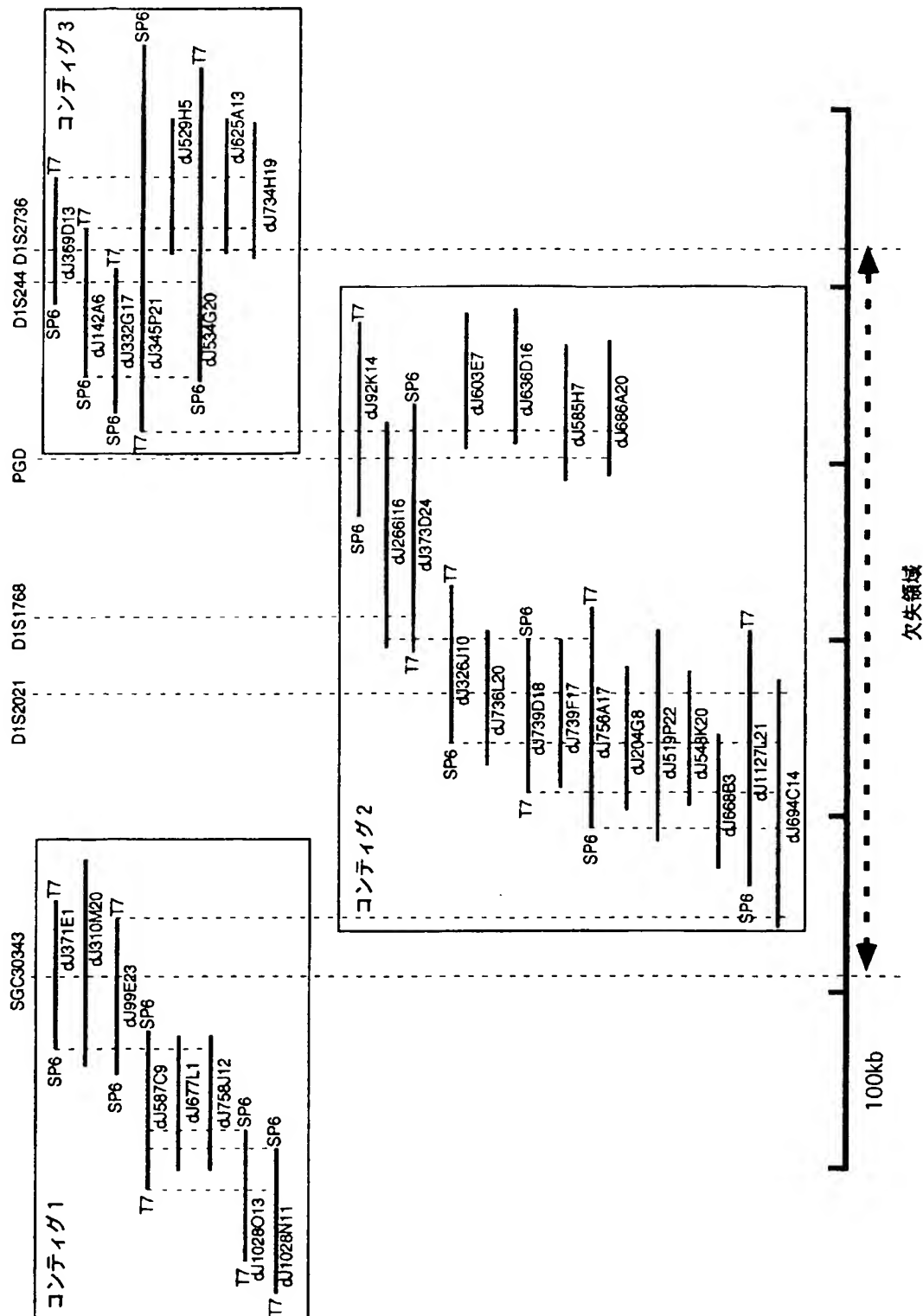
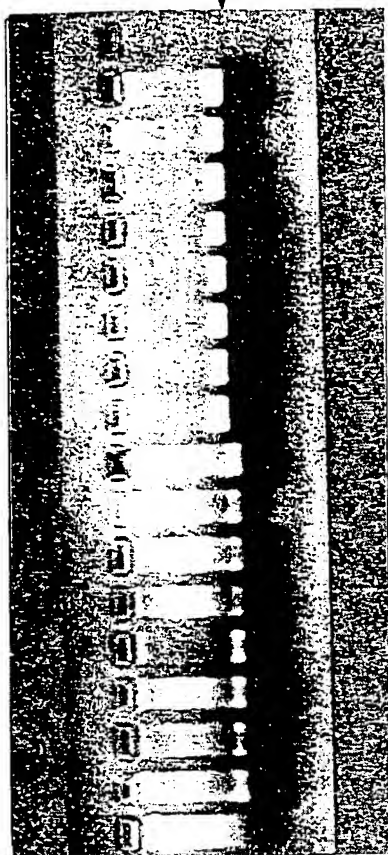


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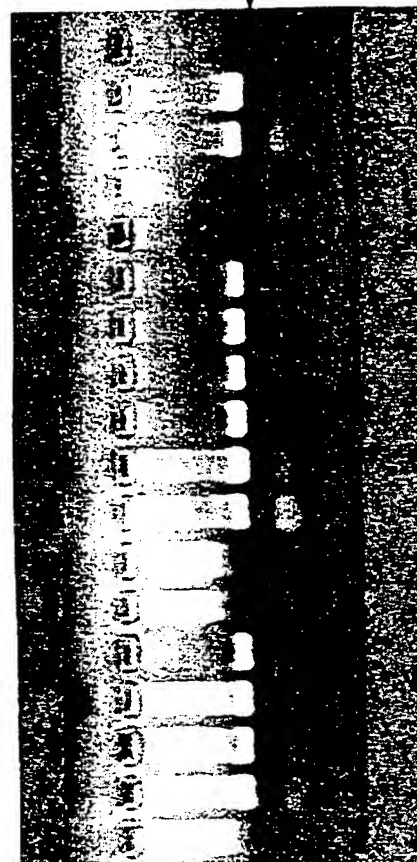


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図6B

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図7

欠失領域の限定

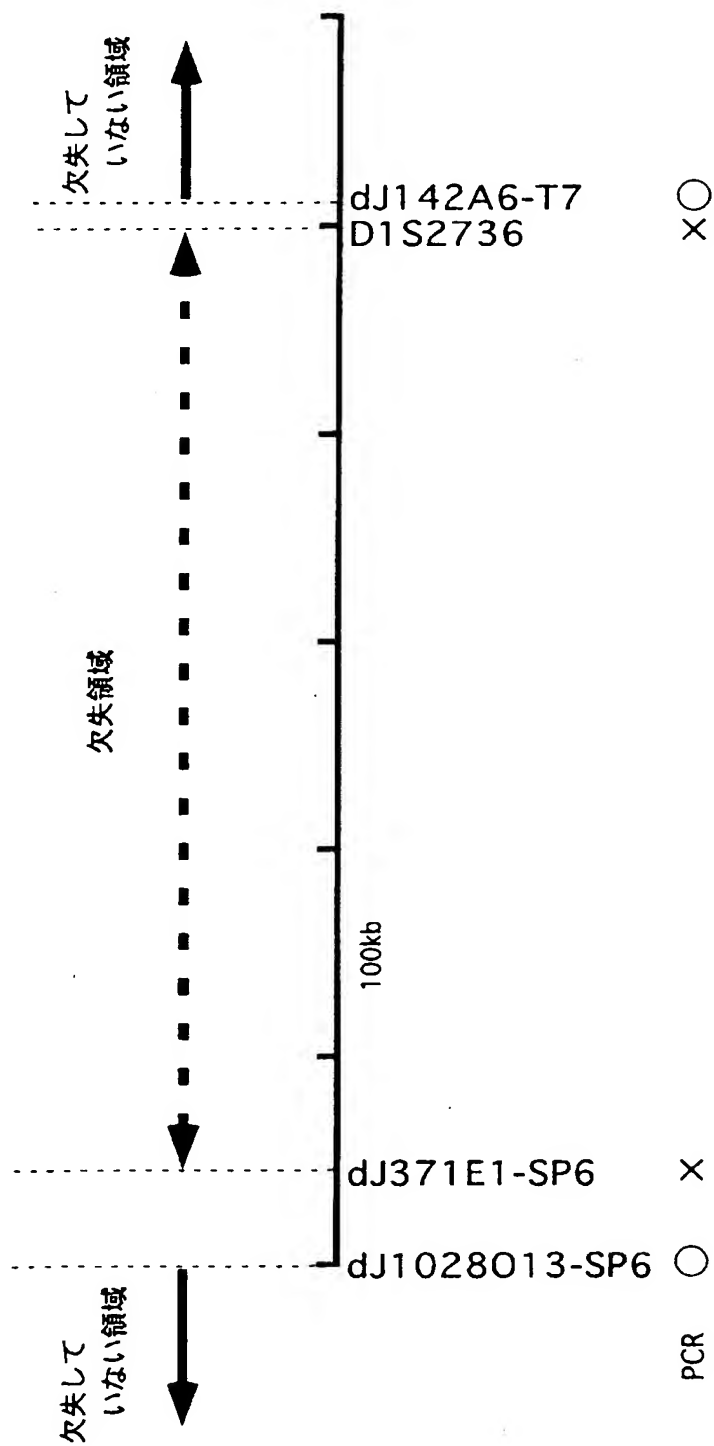


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欠失領域を網羅するPACクロン群1

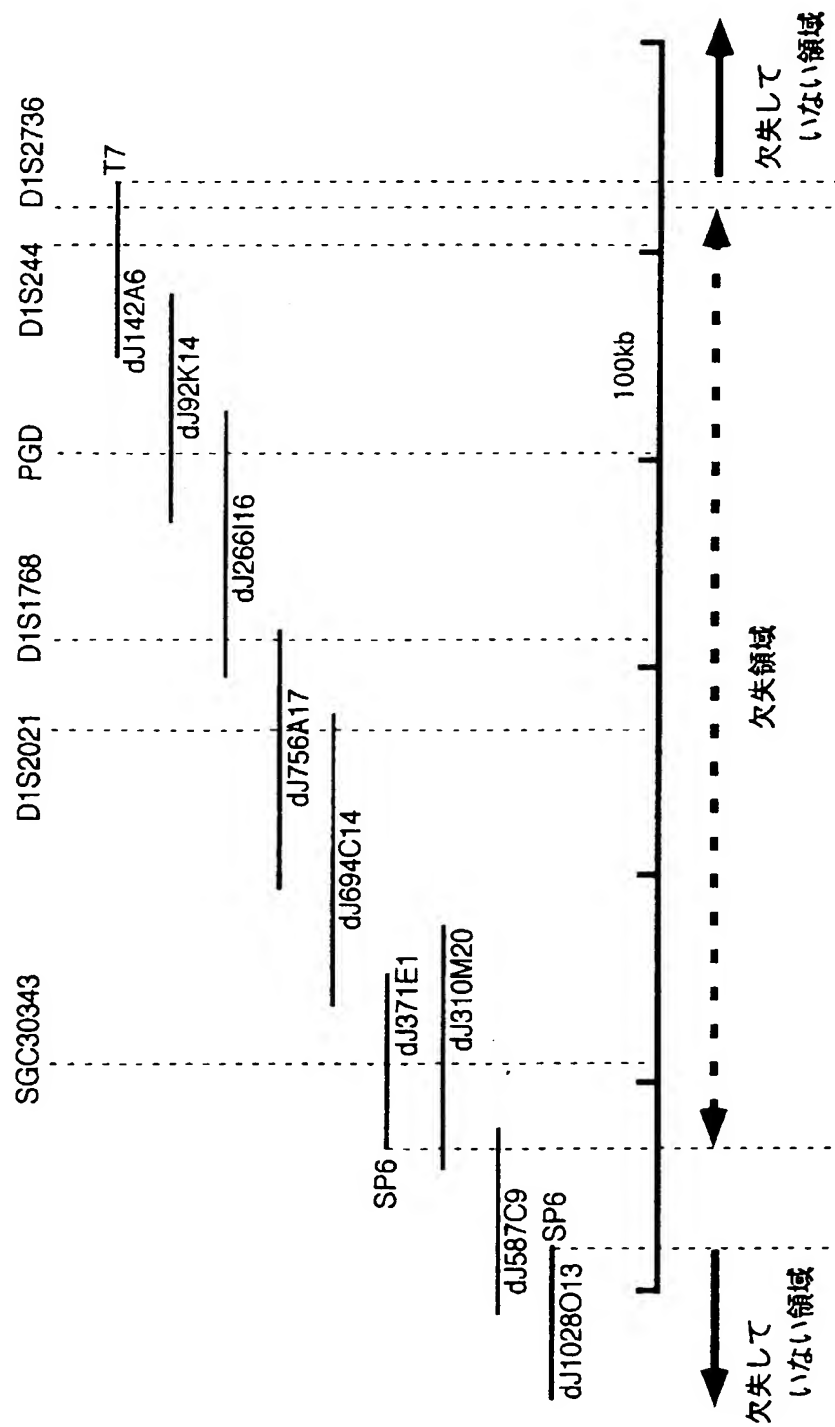
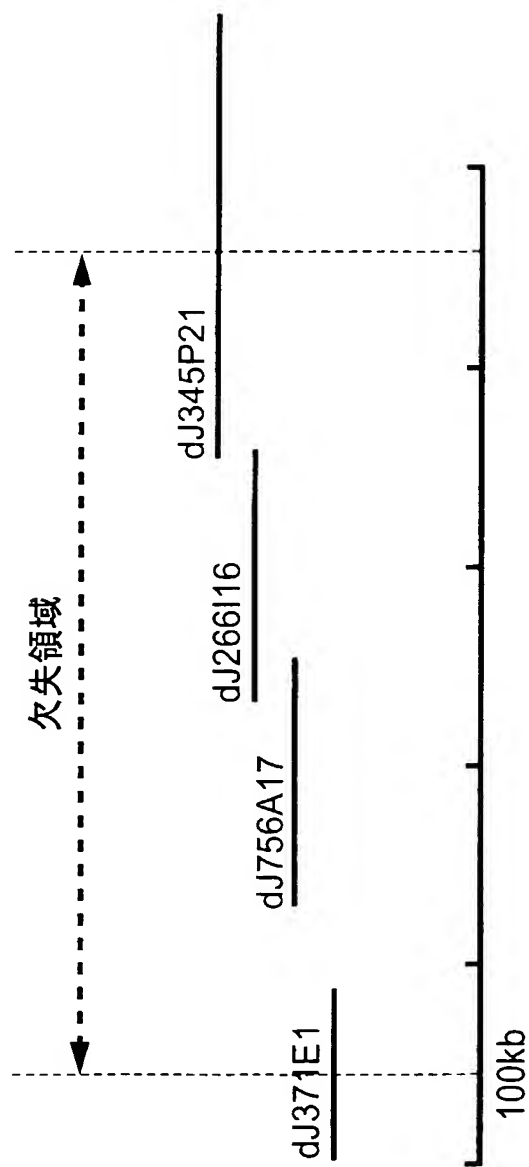


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<211> 1908

<212> DNA

<213> Homo sapience

<400> 46

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<213> Homo sapience

<400> 47

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<211> 5368

<212> DNA

<213> Homo sapience

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<213> Homo sapience

<220>

<223> EST

<400> 49

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<211> 437

<212> DNA

<213> Homo sapience

<220>

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<400> 53

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<211> 302

<212> DNA

<213> Homo sapience

<220>

<223> EST

<400> 54

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<211> 450

<212> DNA

<213> Homo sapience

<220>

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<400> 55

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